

XI. STORAGE & HANDLING

1. Store the kit under normal refrigeration (2° – 8° C or 36° – 46° F). **Do not freeze the kit.**
2. Before conducting the test maintain all kit elements and specimen at room temperature – preferably for 60 – 120 minutes (or 22 minutes at 37° C or 98.6° F). Perform assay at room temperature 20° – 25° C or 68° – 77° F.
3. Avoid spillage and cross-contamination of solutions.
4. Mix reagents by inverting the developing plate several times prior to use.
5. Do not mix reagents from different kits or from different compartments of the same kit.
6. Do not touch the teeth of the ImmunoComb® card.
7. When using developing plate, pierce the cover of each compartment according to the test procedure instructions. Do not remove the aluminum cover of developing plate all at once.
8. The ImmunoComb® kit contains inactivated biological material. The kit must be handled and disposed of in accordance with accepted sanitary requirements.

XII. KIT CONTENTS

Components	90 Test Kit (50MTV103)	900 Test Kit (50MTV130)
ImmunoComb® cards (wrapped in aluminum foil)	3	30
Developing plates	3	30
Positive Control serum vial	1	1
Negative Control serum vial	1	1
Tweezers	1	1
Heparinated capillary tubes	40	May be purchased upon request
CombScale	1	1
Combscore Sheet	1	1
User manual	1	1

XIII. REFERENCES

- Cassel, G. H. (1982). *Rev. Infect. Dis.*, **4**: 518-534.
- Collins, M. J. (1986). *In Hamm, T.E. Complc. VI & Mc Infct in Rodnt to Toxy Rsrch & Test.* Hemisphere Pub., Washington, D.C., pp. 1-24.
- Firer, M. A., et al. (1986). *ImmunoComb® for detect Sendai & coronaviruses & M. pulmonis.* 37th Annual meeting of AALAS, Chicago, IL.

For further assistance please contact your local distributor, or Biogal Laboratories directly by e-mail: info@biogal.co.il or by tel: 972-4-9898605 / fax: 972-4-9898690.

ImmunoComb®

Murine

MYCOPLASMA PULMONIS- RODENT CORONAVIRUS- SENDAI VIRUS ANTIBODY TEST KIT

INSTRUCTION MANUAL

Sufficient for 90 / 900 samples

24.01.11



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I. INTENDED USE OF THE KIT

The Mycoplasma Pulmonis - Rodent Coronavirus - Sendai virus ImmunoComb® Antibody Test Kit is designed for detection of mouse or rat serum antibodies to *Mycoplasma pulmonis* (*M. pulmonis*), Rodent coronaviruses (RCV) and Sendai virus (SV). The kit can be used routinely in facilities with limited capabilities for laboratory testing.

II. WHAT IS THE IMMUNOCOMB® ASSAY?

The ImmunoComb® test is a highly sensitive modified ELISA, which can be described as an enzyme labeled “dot assay” that detects antibody levels in serum or whole blood. The ImmunoComb® test kit contains all necessary reagents for developing the test. IgG antibodies results of all three agents: *M. pulmonis*, RCV and SV tests are obtained within 90 minutes.

III. GENERAL INFORMATION

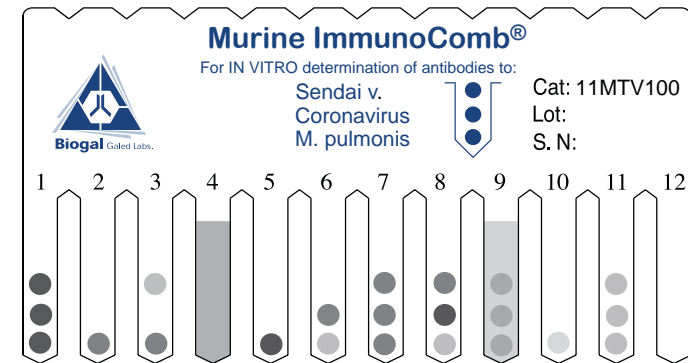
Mycoplasma pulmonis, rodent coronaviruses and Sendai virus are among the main pathogens, which may infect specific pathogen free (SPF) laboratory animals, and interfere with research by distorting biological responses, although not suspected, in the absence of disease. Routine serology has proved to be an efficient tool to reveal the presence of antibodies testifying exposure to specified panels of viral pathogens, using a single serum sample.

Mycoplasma pulmonis – an agent that may cause murine respiratory and genital mycoplasmosis, which impair the usefulness of rodents for research purposes. The *M. pulmonis* antigen may also detect antibodies anti *M. arthritidis*, an antigenically related rodent mycoplasma that causes spontaneous polyarthritis in rats and may also infect mice.

Rodent coronaviruses – a large enveloped RNA group, which includes Mouse Hepatitis virus (MHV), sialodacryoadenitis virus (SDAV) in rats and rat coronavirus (RCV). Mice colonies may be infected by enterotropic MHV strains which usually infest lymphoid tissues, thus may cause significant changes of immune responses or by respiratory strains that may infect internal organs and may cause hepatitis or encephalitis. SDAV and RCV spread from respiratory tract to the lacrimal and salivary glands, causing symptoms like cervical swelling, photophobia and ocular discharge.

Sendai virus - an RNA paramyxovirus of the parainfluenza type 1 group. Mice infected with this virus develop labored breathing and decreased fecundity. In DBA/2 and immune deficient mice, the infection is almost always fatal. The viral caused immunosuppression predisposes the mice to secondary bacterial infections. In endemically infected colonies, many mice may be asymptomatic carriers of the virus. In clinically apparent infections, variable signs may include chattering, respiratory distress to labored breathing, and decreased fecundity in adults. Deaths (possibly whole litters) in neonates and suckling mice can occur due to the weak immune defense at this age.

IX. EXAMPLE OF A DEVELOPED COMB



X. SCORING OF RESULTS

KIT: *M. pulmonis - Coronavirus - Sendai v.* LOT: 070512 COMB ID: 1
 INSTITUTION: Biotech Inc. TEST DATE: Aug., 18, 07 TECHNICIAN: John Doe

		SCORE											
Tooth (well)		1	2	3	4	5	6	7	8	9	10	11	12
Sample ID		10 - 1	10 - 2	10 - 3	10 - 4	11 - 1	11 - 2	11 - 3	12 - 1	12 - 2	12 - 3	C+	C-
Sendai v.		3	0	1	N/S	0	0	2	2	> 1	0	1	0
Corona-virus		3	0	0	N/S	0	2	2	3	> 1	0	1	0
M. pulmonis		3	2	2	N/S	3	1	2	1	> 1	+/-	1	0
Species (circle one)		(M) R	(M) R	(M) R	(M) R	(M) R	M (R)	M (R)	M (R)	M (R)	M (R)	(M) R	(M) R

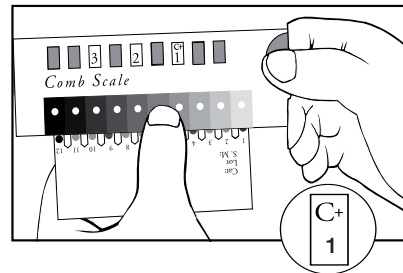
Tooth No.:	Remarks:
4	High background color - Non-Specific invalid test.
9	High background color with higher color spots - Positive.
10	Result <S1 = Negative.
11	Result of the Positive Control.
12	Result of the Negative Control.

VII. READING AND INTERPRETING RESULTS

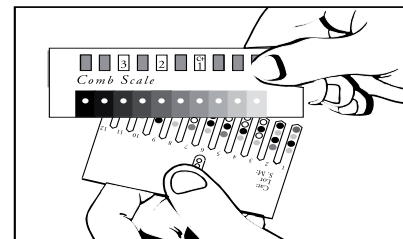
- A test is valid when the Negative Control (C-) tooth shows little or no color (i.e. a score of 0) on all antigen spots, and Positive Control (C+) tooth shows a light purple-grey color, on all 3 spots that matches light color intensity on the CombScale, setting the positive baseline for the test.
- Compare the color result of each antigen spot (on each tooth) to the **same antigen** result on the Positive Control tooth.
- The intensity of the purple-grey color accepted on any of the test spots is scored on a scale of 1 to 3 by comparing any test color result to the Positive control color results, which are scored as 1 and to a color scale (CombScale) (see **section VIII**).
- The absence of detectable antibody typically produces a colorless result that matches the Negative Control and is scored 0.
- If a result above 1 lies between two values, assign the higher score.
- A score of 1 indicates a low antibody titer; score 2 indicates a moderate antibody titer while 3 represents a high antibody titer.
- In **section IX** and **X** you'll find examples of a developed Comb and a completed results form.
- If the area around an antigen spot develops color with a higher intensity than the color of the antigen spot, record the results as NON-SPECIFIC (NS). A NS result does not reveal whether a specimen is antibody positive or negative. Yet, if the color of the spot is darker than the color of the area around it, you may assume that the result is positive but you may not specify the antibody score.

VIII. READING RESULTS WITH THE COMBSCALE

A. When the Comb is completely dry, align it with the calibrated color CombScale provided in the kit. Find the tone of the purple-grey on the CombScale that most closely matches the **Positive Control spot**. Slide the yellow ruler until the C+ mark appears in the window above the color you have found. **Hold the slide in this position during the entire reading.** This step actually calibrates the C+ to S1, which is the “cut-off” point to which test spots will be compared.



B. Read each of the spots separately: Choose the most suitable color and read the titer in the yellow windows.



REMEMBER: A DIFFERENCE OF ONE COLOR LEVEL WILL NOT AFFECT THE DIAGNOSIS!

Another way to read the results is by using the CombScan. This is a software program that utilizes a computer and a twain compatible scanner. When a Comb is placed on the scanner, the program translates the color results into numerical values. The CombScan assists labs in reading ImmunoComb® results and conserving the data, and is supplied free of charge upon request.

IV. HOW DOES THE IMMUNOCOMB® WORK?

- The ImmunoComb® Kit contains 2 main components: comb shaped plastic cards, hereafter referred to as the Comb(s) and multi compartments developing plates divided into compartments A-F that are subdivided into 12 wells. The plate compartments are pre-filled with reagent solutions.
- Each Comb has 12 teeth; noninfectious antigens for *M.pulmonis*, RCV and SV (from bottom to top) are attached to separate sites on each tine to enable simultaneous testing for antibodies to all three agents.
- The first step of the test is to deposit sera or whole blood samples into wells of row A of the multi compartment developing plate along with provided Positive and Negative Control sera.
- Next, the Combs are inserted into the plates, and specific antibodies in test samples, if present, bind to the antigens attached to the Comb teeth.
- The development process takes place in consecutive compartments of the developing plate, and includes 3 incubation periods and some washing steps.
- At the end of the developing process described in section VI, a purple-grey color result is developed in all Positive Control serum spots and in any positive sample tested spot. The intensity of the colored product is proportional to the titer of specific antibodies in the specimen.
- A negative result indicates that the mouse/rat has not been exposed to the tested pathogen(s)!
- Any positive valid result indicates exposure to the relevant pathogen.

V. LIMITATIONS OF THE KIT

The Murine ImmunoComb® Kit is subject to limitations inherent in any serologic assay, including false negative or false positive results.

A false negative result occurs when the specific antibody level is below that which is detectable in the test. This is most likely to happen when rodents are tested shortly after the onset of infection. Seroconversion is not detectable by the ImmunoComb® or by any other serological assay before about two weeks post virus exposure. A false negative result is also likely to occur when immunodeficient rodents are tested.

A false positive result may occur in the ImmunoComb® assay if substantial amounts of antibody in a specimen bind non-specifically to an antigen spot for reasons that are not clearly understood.

False positive as well as **non-specific (NS)** results may occur because specimens are of poor quality due to improper collection, preparation and/or storage or were collected from rodents with autoimmune disease or that had been inoculated with transplantable tumors or other biological materials.

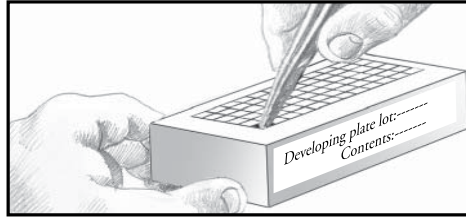
One should suspect that positive results are not authentic if they are unexpected, not reproducible or found only in a low percentage of animals tested from a colony. It is strongly recommended that NS and questionable results be corroborated by retesting specimens and testing additional mice with the ImmunoComb® or alternate diagnostic methods. Specimens for testing should be selected and results should be interpreted with the above considerations in mind.

VI. STEP BY STEP WITH IMMUNOCOMB®

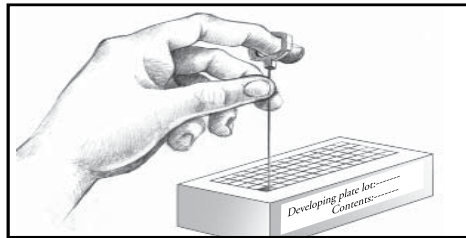
Perform assay at room temperature 20°-25° C (68° - 77°F)

(1) Obtain blood samples from mouse/rat.

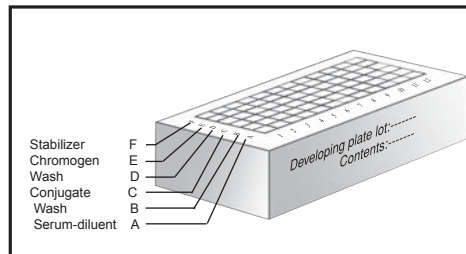
(2) Use the tweezers to pierce the protective aluminum cover of **row A**. One well for each specimen. Use a pipette or capillary tube. For testing whole blood use 10 µl. For testing serum/plasma use 5 µl.



(3) Deposit a sample into a well in **row A** that contains serum diluent. Raise and lower pipette/piston plunger several times to achieve mixing. Each plate may be used to test **individual or any number of samples up to 10**, by breaking off the desired number of teeth from the Comb and using the corresponding column of wells in the developing plate.

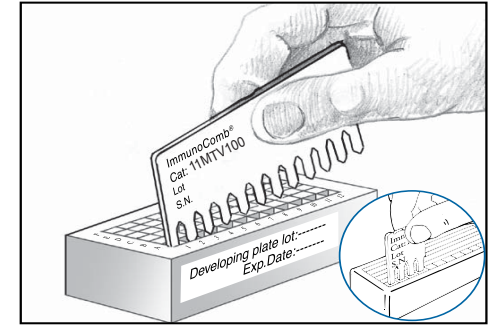


(4) For control sera open the next two consecutive wells. Take 5µl **Positive Control** serum (C+) and insert into well A next to the last sample. Mix the serum in the well. Do the same with the **Negative Control serum** (C-) in next well. Each run should include Positive Control and Negative Control wells/teeth.

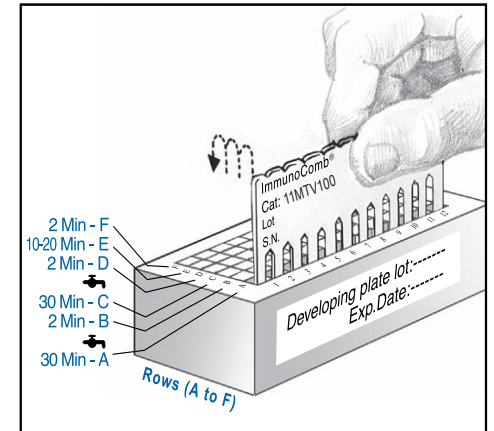


(5) Remove one Comb from its protective wrapping and insert it (printed side facing you) into **Row A**. Incubate for **30 minutes**. To improve mixing, gently move Comb **up and down** at the start of each incubation (each row). Repeat this motion at least twice in all of the remaining rows.

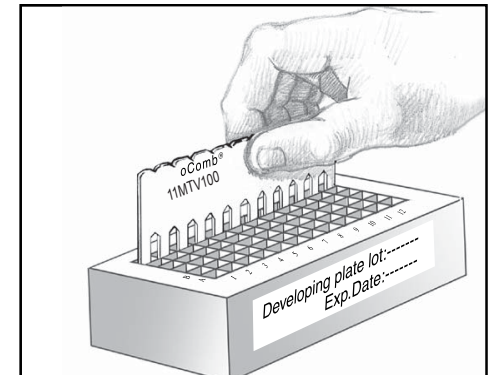
When using a partial Comb, cut the number of teeth needed including Positive and Negative Controls. Keep the remaining unused teeth sealed in its original envelope for further use. In each further step, open and use only the corresponding wells in the developing plate.



(6) Use tweezers to pierce the foil of the next wells (**row B**). Wash Comb under cool tap water and insert Comb into **row B** for **2 minutes**. Before transferring Comb into next row, pierce the foil of the next needed compartments of **row C**. Gently shake off excess liquid from Comb teeth onto a tissue and insert Comb into **row C** (which contains diluted anti rat & mouse conjugate) for **30 minutes**. Use tweezers to pierce the foil of the next wells (**row D**). Wash Comb under cool tap water and insert Comb into **row D** for **2 minutes**. Before transferring Comb into next row, pierce the foil of the next needed compartments of **row E**. Gently shake off excess liquid from Comb teeth onto a tissue and insert Comb into **row E** (which contains chromogen) for **10-20 minutes**.



(7) Use tweezers to pierce the foil of the next wells (row F). Upon completion of color development in row E the positive control tooth should develop a light purple-grey color. Move Comb to **row F** for **2 minutes** for color fixation. Take Comb out, shake off excess liquid and let it dry.



(8) Record the results immediately on the Murine ImmunoComb® Result form, and file it. You may keep the developed dry Comb as a record.